Mechanistic Evaluation of Factor IX-Padua Activity in Chromogenic FIX and Thrombin Generation Assays

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Background: Our gene therapy trial featuring AAVS3 FIX-Padua (FLT180a) is targeting FIX-Padua expression levels that functionally cure haemophilia B. Recent data shows that FIX-Padua activity (FIXp:C) assay results can vary by up to 3-fold depending on the assay used. Gene therapy clinical outcomes can vary substantially over a 3-fold FIX:C range emphasizing the need to understand mechanisms causing FIX assay discrepancy and how FIXp:C maps on to wild-type FIX activity (FIXwt:C). A thorough understanding of FIXp:C will help identify the appropriate expression target in clinical trials and inform on which assays are most suitable for monitoring gene therapy patients.

Aims: We scrutinized one-stage and chromogenic FIX assays to identify mechanisms causing assay discrepancy and sought to determine how FIXp:C relates to FIXwt:C.

Methods: We spiked FIXwt or FIX-Padua into haemophilia B plasma to yield various FIX activities. Samples were used to evaluate the impact of increasing FX on chromogenic FIX results and the difference between FIXp:C or FIXwt:C in tissue factor-initiated thrombin generation assays.

Results: Chromogenic assays contain normal FVIII levels, but sub-physiological concentrations of FX. Increasing FX in chromogenic assays dose-dependently increases FIXp:C up to 2-fold but has no effect on FIXwt:C levels. Interestingly, after normalizing FX in chromogenic assays, FIXp:C results are similar to one-stage assay results. In thrombin generation assays, given FIX:C levels, whether supplied by FIX-Padua or FIXwt, yield similar thrombin generation parameters.

Conclusions: Our data indicate that FX is limiting in chromogenic assays when measuring FIXp:C and supplementing FX can restore FIXp:C to levels measured with one-stage clotting assays. Altogether, our data suggests that one-stage FIX assays may provide a better estimate of FIXp:C and this activity is similar to FIXwt:C in driving physiologically relevant thrombin generation.

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